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Chromosomal evolution in the South American Riodinidae (Lepidoptera: Papilionoidea)

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We give the haploid chromosome numbers of 173 species or subspecies of Riodinidae as well as of 17 species or subspecies of neotropical Lycaenidae for comparison. The chromosome numbers of riodinids have thus far been very poorly known. We find that their range of variation extends from n = 9 to n = 110 but numbers above n = 31 are rare. While lepidopterans in general have stable chromosome numbers, or variation is limited at most a subfamily or genus, the entire family Riodinidae shows variation within genera, tribes and subfamilies with no single modal number. In particular, a stepwise pattern with chromosome numbers that are about even multiples is seen in several unrelated genera. We propose that this variation is attributable to the small population sizes, fragmented populations with little migration, and the behavior of these butterflies. Small and isolated riodinid populations would allow for inbreeding to take place. Newly arisen chromosomal variants could become fixed and contribute to reproductive isolation and speciation. In contrast to the riodinids, the neotropical Lycaenidae (Theclinae and Polyommatinae) conform to the modal n = 24 that characterizes the family.

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Metalmark butterflies (Riodinidae) have a cosmopolitan but highly uneven distribution. In a total of some 1300 species worldwide, about 1200 are found exclusively in the Neotropics (DeVries 1997; Hall and Harvey 2002a, 2002b, 2002c). In this region they come in myriad color patterns and shapes, and range in size from medium to small. This extraordinary diversity of riodinids could be explained in part through Batesian (and possibly Müllerian) mimicry that appears to be more prevalent in this family than in any other similar-sized family of insects. Brown (1993a) describes the extent of mimicry in riodinids, but the details of its evolution seem to be relatively little known (DeVries 1997). The taxonomy of some tribes and genera has been resolved (Hall 2002; Hall and Harvey 2002a), but many points remain open (DeVries 1997; Callaghan and Lamas 2004).

There seems to be a consensus (Campbell et al. 2000; Campbell and Pierce 2003; Vane-Wright 2003; Wahlberg et al. 2005) that the riodinids are most closely related to the lycaenid butterflies, and that the nymphalids are the closest relatives of this riodinid-lycaenid clade. Lycaenids are a large family with about 5000 species that account for about one fourth of all Papilionoidea (Pierce et al. 2002). The diversity of lycaenids is greatest in Africa.

In his last book 'Modes of speciation' M. J. D. White (1978, p. 73) chose the butterflies to illustrate the distribution of chromosome numbers in a well-studied group. He shows a histogram that includes the chromosome numbers of 738 species worldwide. In addition, White (1978) pointed out that the only family that has a well-marked type number of its own is Lycaenidae in which the numbers n = 23 and 24 are common, but that spectacular increases and decreases have occurred in certain lineages of this family. In fact, *Polyommatus (Plebicula) atlantica* with n = 221–223 has the highest chromosome number observed in a non-polyploid Metazoan. White (1978) showed separately the distribution of chromosome numbers for the families Lycaenidae and Riodinidae in the histogram mentioned above. A perusal of this histogram shows that there are very few riodinids among the 738 species of butterflies included. Evidently the riodinids are a large but cytogenetically little-known group of butterflies.

The chromosomes of lepidopterans are holokinetic (or nearly holokinetic, Brown et al. 2007a, 2007b) chromosomes, a circumstance that can be thought to facilitate chromosomal rearrangements since even small fragments can attach to spindle fibers at cell division. More studies are needed here, since e.g. Hipp et al. (2010) have shown that both fusions and fissions of holokinetic chromosomes restrict gene flow in plants with such chromosomes.

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Many groups of butterflies are characterized by a great stability of chromosome numbers. In addition to the lycaenids mentioned by White (1978), the papilionids have, with a few exceptions, n = 30 (Emmel et al. 1995). Nymphalidae have clearly the lepidopteran modal n = 29–31 as the ancestral condition (Brown et al. 2007b). Different Neotropical groups show an evolution away from this modal number: Morphini have a strong modal n = 28–29, while the Neotropical Satyrinae (sensu lato) show only a weak modal of n = 29 (Brown et al. 2007a). The primitive Heliconiini have n = 31, but the derived and large genus *Heliconius* is stabilized at n = 21 (Brown et al. 1992). Finally Ithomiini show extensive variation with a clear modal number at n = 14–15 (Brown et al. 2004). Stekolnikov et al. (2000) have also reviewed Nymphalid chromosome numbers worldwide.

We give here the chromosome numbers for 173 South American riodinid species and subspecies and try to evaluate whether evolutionary patterns can be discerned. In particular, we wish to find out whether there is a modal number and if not, which factors drive the diversity. As a reference we use the results obtained for nymphalids (Brown et al. 1992, 2004, 2007a, 2007b) and lycaenids, extensively covered in the literature (Robinson 1971; White 1978). The overall aim is to cast light on the relationships and evolution in a cytogenetically little studied butterfly family.

MATERIAL AND METHODS

Keith Brown collected the butterflies in different parts of South America during the 1970s and 1980s. The (often generalized) collection localities are given in the Tables in the Results section.

We prepared the gonads of the butterflies as described in detail by Brown et al. (1992) and stored them for variable lengths of time until subjected to sectioning, staining and microscopy (as described by Suomalainen and Brown (1984)).

Barbara von Schoultz did the practical laboratory work in Helsinki in the 1980s up to the year 1994. The late Dr. Esko Suomalainen of the Department of Genetics of the University of Helsinki, Finland, checked the chromosome number counts.

Since about 40% of the material studied can not at present reliably be assigned to species, we give the voucher number for each specimen here. The exact collecting localities, dates and voucher specimens are stored at the Museu de História Natural of the Universidade Estadual de Campinas, SP, Brazil, while the original laboratory notebooks and chromosome slides of the material studied in Finland are at the Finnish Museum of Natural History, University of Helsinki, Finland. We have also included earlier chromosome counts reported by Maeki and Remington (1960), de Lesse (1967, 1970), de Lesse and Brown (1971) and Wesley and Emmel (1975).

RESULTS

We give the haploid chromosome numbers for 173 South American Riodinidae taxa in Table 1 and 2. The species are arranged according to the subfamily, tribe and subtribe division of Callaghan and Lamas (2004), but the order of species within each category is alphabetical in Table 1. Table 2 shows the chromosome numbers for taxa that could not be reliably assigned to species.

An inspection of the Tables shows that there is extensive variation in chromosome numbers between (and possibly within) species. The lowest is n = 9 (*Calephelis aymaran*) and the highest n = about 110 (*Mesosemia* sp.). The sample sizes are in general small. However, when several samples from different populations are available, there often is no variation within the taxon. Some species and populations showed widely different chromosome numbers, suggesting the possibility of cryptic species not recognized or polymorphism. For example, *Emesis mandana* and *Necyria bellona* show variation within a single population and *Melanis aegates albugo* shows variation among populations. Some individuals have different numbers like n = 28–29. Such a phenomenon is most likely attributable to variation within an individual.

In addition to the pattern of variation in which populations belonging either to the same or related species differ by a few chromosomes there is evidence for a different stepwise pattern in which either populations of a species or species that belong to the same genus have chromosome numbers that represent about even multiples of each other. Among the riodinids *Calydna thersander* has both n = 33 and n = 64; the genus *Calephelis* has species with numbers ranging from n = 9 and n = 10 to n = 23 to 45. Other such cases include *Eurybia nicaeus* with n = 29 and *E. nicaeus paula* with n = 14.

There are a few genera with what appears to be a stable chromosome number, for example, almost all *Nymphidium* have n = 31. The 'primitive' subfamily Euselasiinae and the tribe Eurybiini have the numbers close to n = 28–29 most common, but some species may have a number about half of that. The other tribes have highly variable chromosome numbers. The tribe Riodinini shows three pairs of common numbers: n = 15–16, 20–21 and 27–28 with relatively few counts in between. The range of overall variation in Riodinidae is in general between n = 14 and n = 31.

Table 1. *Haploid chromosome numbers for species and recognized additional subspecies of South American riodinids. The taxon name used in the original reference is in parentheses. A comma between chromosome numbers indicates different individuals, a dash indicates uncertain alternative numbers within an individual.*

Voucher code	Genus	Species	n =	No. studied pop./ind.	Locality
Subfamily EUSELASIINAE					
Tribe Euselasiini					
314	*Euselasia*	*clesa*	29	1/1	ES
335		*eucerus*	28	1/1	ES
260		*eusepus*	29	1/1	ES
304		*fervida*	29	1/1	ES
1117		*gelanor*	28	1/1	EB
419		*melaphaea*	12	1/1	MT
476		*mys cytis*	28	1/1	MT
399		*praeclara*	29	1/1	MT
252		*thucydides*	27	1/1	ES
316		*utica*	17	1/1	ES
1452	*Hades*	*noctula*	20	1/1	CC
Subfamily RIODININAE					
Tribe Mesosemiini					
Subtribe Mesosemiina					
428, 438, 455	*Mesosemia*	*bella*	21, 23	1/1, 2/2	MT; MT2
Wesley and Emmel 1975 (TR-118)		*lamachus (methion)*	18	1/1	Trinidad
189		*melpia*	20	1/2	DF
464		*melpia vaporosa* (?)	ca 46	1/1	MT
577		*metope*	26	1/1	PB
951, 866		*mevania mimallonis*	43, 45	1/1, 1/2	VC, VV
Subtribe Napaeina					
261	*Cremna*	*alector*	24	1/1	ES
414		*cuyabaensis*	43	1/1	MT
158	*Eucorna*	*sanarita*	13	1/1	RJ
310	*Napaea*	*eucharila*	22	1/1	ES
298		*orpheus*	26	1/2	ES
Tribe Eurybiini					
1146	*Alesa*	*prema*	21	1/1	MG
1120, 1042	*Eurybia*	*dardus*	14	2/2	EB, EE
442, 400, 445, 751, 907		*dardus annulata*	28, 29, 32	1/1, 2/3, 1/1	MT; MT, PA; VV
974		*franciscana* ssp.	14	1/1	WE
1541		*halimede*	29	1/1	BA
de Lesse and Brown 1971		*halimede passercula (elvina tephrias)*	29	1/1, 1/1	DF, MG
de Lesse and Brown 1971		*misellivestis (dardus misellivestis)*	13	1/2	MG
de Lesse and Brown 1971		*molochina hyacinthina*	30	1/1, 1/1	RJ
743, 1059		*nicaeus*	29	1/1, 1/1	EE, PA
de Lesse and Brown 1971		*nicaeus* (f. *paula*)	14	1/1	DF
1250		*nicaeus* ssp. (violet HW)	29	1/1	RO
Tribe Riodinini					
380, 454	*Amarynthis*	*meneria*	20	2/3	MT2
307	*Ancyluris*	*aulestes pandama*	27	1/2	ES
1229		*meliboeus*	27	1/1	RO
311	*Baeotis*	*hisbon*	28	1/1	ES
267		*melanis* (?)	15	1/2	ES
de Lesse 1967	*Barbicornis*	*basilis mona*	30	1/2	Argentina
529	*Calephelis*	*aymaran*	9	1/1	BA

(Continued)

Table 1. (Continued).

Voucher code	Genus	Species	n =	No. studied pop./ind.	Locality
de Lesse 1967		*candiope (Charmona c.)*	23	1/2	Colombia
de Lesse 1967		*nilus*	10	1/1	Bolivia
Maeki and Remington 1961		*virginiensis*	45	1/1	Florida
270	*Chalodeta*	*chelonis*	16	1/1	ES
de Lesse 1967		*theodora*	15	1/1	Argentina
708	*Charis*	*cadytis*	27–28	1/1	MG
175, 456	*Chorinea*	*amazon*	21, 27	1/1, 1/1	MG, MT
519		*octauius*	20	1/3	MT
de Lesse and Brown 1971	*Detritivora*	*zama* [*Charis (gynaea?) zama*]	20	1/1	DF
562	*Isapis*	*agyrtus*	27	1/1	PE
450	*Lasaia*	*agesilas*	22 (?)	1/2	MT
452	*Lyropteryx*	*apollonia*	25	1/1	MT
de Lesse 1967	*Melanis*	*aegates aegates (Lymnas a.)*	16	1/2	Argentina
241, 447, 440, 427		*aegates albugo*	20, 21, 27	1/1, 1/1; 1/1; 1/1	MG, MT; MT; MT
1188		*aegates/hillapana*	21	1/1	MT
224, 829		*electron electron*	19, 21	1/2, 1/1	MG, RG
422		*marathon*	34	1/3	MT
569, 443		*smithiae*	16, 31	1/1, 1/2	PE, MT
437		*xenia ambryllis*	31	1/2	MT
179	*Metacharis*	*lucius*	11	1/1	GO
1471a, 1471b	*Necyria*	*bellona*	21, 27	1/1, 1/3	EE
de Lesse and Brown 1971	*Panara*	*jarbas (thisbe)*	15, 16	1/1, 1/1	MG
239		*ovifera*	26	1/1	RJ
de Lesse and Brown 1971, 423	*Rhetus*	*periander (arthuriana)*	15	1/1, 1/2, 1/1	GO, MT
477, 792	*Riodina*	*lysippus*	14	1/3, 1/2	MT, RR
de Lesse 1967	*Siseme*	*neurodes*	17	1/1	Bolivia
295	*Syrmatia*	*nyx*	21	1/1	ES
Tribe Symmachiini					
439	*Mesene*	*monostigma*	24	1/1	MT
563		*phareus*	27	1/1	PE
793	*Phaenochitonia*	*cingulus*	8–10	1/1	RR
513		*rufilinea*	10	1/3	MT
de Lesse and Brown 1971	*Pirascca*	*sagaris satnius (Phaenochitonia s. s.)*	20	1/1	GO
1595	*Symmachia*	*accusatrix*	22	1/1, 1/1	ES
319		*menetas*	27	1/1	ES
Tribe Helicopini					
812, 3317	*Helicopis*	*cupido*	20	2/2	GY, AV
1028		*gnidus*	16	1/1	EE
Tribe *INCERTAE SEDIS*					
1366	*Calydna*	*lusca*	40	1/1	ES
583, 1187		*thersander*	33, 64	1/1, 1/1	PE, MT
1118	*Echydna*	*chaseba* (?)	26	1/1	EB
de Lesse 1967	*Emesis*	*angularis*	ca 34	1/1	Bolivia
de Lesse 1967		*cypria*	17	1/1	Ecuador
463		*lucinda lucinda*	29	1/1	MT
910		*lucinda* ssp.	25	1/1	VV
425a,b,c		*mandana*	11, 21, 27	1/1, 1/1, 1/1	MT

(Continued)

Table 1. (Continued).

Voucher code	Genus	Species	n =	No. studied pop./ind.	Locality
1446		*ocypore* ssp.	15	1/1	CC
654		*tenedia*	14	1/1	SC
de Lesse 1967	*Imelda*	*mycea glaucosmia*	21	1/1	Ecuador
Tribe Nymphidiini					
Subtribe Aricorina					
de Lesse 1967	*Aricoris*	*chilensis (Hamearis c.)*	31	1/1	Argentina
de Lesse 1967, 797		*epulus (Hamearis e.)*	29, 28–30	1/1, 1/1	Argentina, RR
1181		*middletoni*	34 (?)	1/1	MT
Subtribe Lemoniadina					
750	*Juditha*	*azan majorana*	13	1/1	PA
479		*azan*?	31	1/1	MT
1265, 673	*Lemonias*	*zygia*	24, 25	1/1, 1/1	RO, RJ
726	*Synargis*	*abaris*?	24	1/2	PA
1161		*brennus*	ca 20	1/1	DF
de Lesse and Brown 1971; 659		*calyce*	17	1/1, 1/1	GO
796		*orestessa* (?)	18	1/2	RR
1577		*phliasus*	17	1/1	SP
802		*pittheus*	27	1/1	RR
de Lesse and Brown 1971	*Thisbe*	*irenea*	ca 15	1/1	RJ
Subtribe Nymphidiina					
1004	*Adelotypa*	*huebneri*	20	1/1	EE
300		*leucophaea*	30	1/2	ES
744	*Calospila*	*lucianus* ssp.	ca 30	1/1	PA
655, 672	*Menander*	*menander nitida*	28–29, 29	1/1, 1/1	SC, RJ
619	*Nymphidium*	*acherois*	31	1/1	PE
421		*caricae*	31	2/2	MT
de Lesse and Brown 1971		*leucosia*	31	1/1	DF
481		*mantus*	31	1/2	MT
1598		*molpe* (?)	31	1/1	BA
Subtribe Theopina					
497	*Theope*	*acosma*	16	1/3	MT
605		*foliorum*	29	1/1	PE
1551		*foliorum* (?)	32	1/1	BA
Tribe Stalachtini					
Rio 10	*Stalachtis*	*magdalena*	19	1/1	VC
de Lesse and Brown 1971		*phlegia*	28	1/2	DF
de Lesse and Brown 1971		*phlegia susanna*	36	1/1	RJ

Localities are grouped by region; a number at the end of the locality code indicates that more than one population has been sampled within this region. Locality codes: AM = Amazonas (northwestern Brazil), AV = Amazonas, (southern Venezuela), BA = Bahia (eastern Brazil), CC = Chocó (western Colombia), DA = Darien (southern Panama), DF = Brasília (central Brazil), EB = eastern Bolivia, EE = eastern Ecuador, ES = Espírito Santo (eastern Brazil), GO = Goiás (central Brazil), GY = French Guyana, MG = Minas Gerais (central Brazil), MT = Mato Grosso (central Brazil), OX = Oaxaca (southern Mexico), PA = Pará (northern Brazil), PB = Paraíba (northeastern Brazil), PE = Pernambuco (northeastern Brazil), PT = Putumayo (southern Colombia), RG = Aragua (northern Venezuela), RJ = Rio de Janeiro (southeastern Brazil), RO = Rondônia (western Brazil), RR = Roraima (northern Brazil), SC = Santa Catarina (southern Brazil), SP = São Paulo (southeastern Brazil), TV = Táchira (western Venezuela), VC = Valle de Cauca (western Colombia), VV = Villavicencio, Meta (eastern Colombia), WE = western Ecuador.
In addition, certain details on fore or hind wings are abbreviated as FW or HW.

We also give the chromosome numbers for 17 Neotropical lycaenids (Theclinae and Polyommatinae) for comparison in Table 3. The names of species follow the checklist of Robbins and Lamas (2004). Among the theclines, *Eumaeus minyas* has both n = 24 and n = ca 45. With three exceptions the Neotropical Lycaenidae have n = 24, characteristic for this family elsewhere.

Table 2. *Chromosome numbers for riodinids that have not been reliably assigned to species. Certain details on fore or hind wings are abbreviated as FW or HW. Otherwise as in Table 1.*

Voucher code	Genus	Species	n =	No. studied pop./ind.	Locality
Subfamily EUSELASIINAE					
Tribe Euselasiini					
970	*Euselasia*	sp. nr *eusepus*	29	1/2	WE
1278		sp. nr *cafusa*	28	1/1	RO
1210		sp. (large)	18	1/1	MT
Tribe Mesosemiini					
Subtribe Mesosemiina					
1517	*Mesosemia*	sp. nr *metuana*	26	1/1	EE
1023, 1514		sp. nr *mevania*	44	2/2	EE2
1143		sp. (blue-lined, light)	11	1/1	RO
778		sp. (like large *metope*)	25	1/1	AM
1119		sp.	26	1/1	EB
1283		sp. (white HW border)	27	1/1	MT
3263		sp.	41	1/1	RR
1397		sp. (large, very blue)	43–44	1/1	VC
785		sp. (blue lines)	ca 110 (two large chr.)	1/1	AM
Subtribe Napaeina					
1052	*Ithomiola*	sp.	25	1/2	EE
Tribe Eurybiini					
1021	*Alesa*	sp. (blue-black)	19	11	EE
846	*Eurybia*	*franciscana* (?)	17	1/1	TV
1082a		sp. (small)	14	1/2	EE
1082b		sp. (small)	26	1/1	EE
1444		sp. (large, yellow on HW)	29	1/1	CC
1526		sp. (eye, violet HW)	29	1/1	WE
1213		sp. (violet HW)	29–30	1/1	MT
Tribe Riodinini					
1440	*Ancyluris*	sp. (narrow shorter band)	27	1/1	CC
1382		sp.	28	1/1	EE
1496		sp.	28	1/1	EE
1230		sp. (straight caudal red)	31	1/1	RO
1015	*Caria*	sp. (red under FW)	16	1/1	EE
914	*Charis*	sp. (dark)	16	1/1	PT
652a		sp.	18	1/1	SC
652b		sp.	25	1/1	SC
1266		sp. (blue ventrally)	29	1/1	RO
1064	*Crocozona*	sp.	23	1/1	EE
1038	*Ithomeis*	sp. (large, orange-tipped)	18 (?)	1/1	EE
475	*Melanis*	sp. (‘white spot’)	12	1/2	MT
697		sp. (*albugo*?)	20	1/2	ES
1466		sp. (orange HW border)	33	1/1	EE
554		sp.	38	1/2	PE
634		sp.	15	1/2	BA
606		sp.	25	1/1	PE
1476	*Rhetus*	sp. (white lines under)	15	1/1	EE
1380		sp.	15	1/1	EE
Tribe Symmachiini					
404	*Mesene*	sp. (two-dot)	15	1/1	MT
441	*Symmachia*	sp.	14	1/1	MT
651		sp. (dark)	18	1/1	SC
Tribe *INCERTAE SEDIS*					
327	*Argyro-grammana*	sp. (red)	16	1/1	ES

(Continued)

Table 2. (Continued).

Voucher code	Genus	Species	n =	No. studied pop./ind.	Locality
302		sp.	30	1/1	ES
689	*Emesis*	sp.	45	1/1	RJ
Tribe Nymphidiini					
Subtribe Lemoniadina					
564	*Synargis*	sp. nr *phliasus*	20	1/1	PE
1436		sp. (orange half HW)	15	1/1	CC
Subtribe Nymphidiina					
781	*Adelotypa*	sp. (russet FW, white HW)	21	1/1	AM
1112		sp. (dark)	ca. 30	1/1	EB
1435	*Calospila*	?sp. (small pattern)	30	1/1	CC
763	*Menander*	sp. (very blue)	18	1/1	AM
518		sp.	18	1/4	MT
779	*Nymphidium*	sp. nr *cahcrus*	21	1/1	AM
733		sp. (light orange on borders)	31	1/1	PA
515		sp.	31	1/2	MT
Subtribe Theopina					
1149	*Theope*	sp. (blue and grey)	16	1/1	MG
UNKNOWN					
1361	Riodinid	like *Callicore (Orimba?)*	27	1/1	EE
1467		sp. (small, blue, short white band)	27	1/1	EE

DISCUSSION

The results show that the riodinids have widely variable chromosome numbers, while the Neotropical lycaenids largely conform to the modal n = 24 that characterizes the family (White 1978). The riodinids do not appear to have a distinct modal number. Very few (six at most in this study) have the n = 23 or 24 that characterize the lycaenids (Fig. 1).

The most common numbers among riodinids are n = 27, 29 and 31. The latter two belong to the modal n = 29–31 of butterflies (White 1978; Brown et al. 2007a, 2007b). The distribution of these numbers is, however, uneven and it is difficult to discern a pattern at all. Nevertheless, the most primitive Euselasiinae have n = 28–29, while the highly advanced genus *Nymphidium* is nearly stabilized at n = 31. Maeki and Ae (1968a, 1968b) give chromosome numbers for three representatives of the small Old World subfamily Hamerarinae (Nemeobiinae): *Abisara burnii etymander* (from Taiwan) has n = 30, *Abisara echerius echerius* (from Hong Kong) n = 31, *Zemeros flegyas flegyas* (from Hong Kong) n = 31.

The over-all distribution of riodinid chromosome numbers with no modal one is unique among the Lepidoptera. The nymphalids are the sister group of the evidently monophyletic Lycaenidae and Riodinidae (Campbell et al. 2000). The satyroids have a weak modal n = 29 with many numbers lower than that and rather few higher than the modal numbers (Brown et al. 2007a) but even they include many groups with stable chromosome numbers. Ithomiinae, another variable nymphalid subfamily, seem to have originated from a lineage that already has had the nymphaline modal, n = 31 (Brown et al. 2007b) divided by two through what appears to have been pairwise fusions of all chromosomes, with about n = 14 as a predominant number.

If the Ithomiinae have had their chromosome number halved, then such a process runs riot among the riodinids. They, and *Eumaeus* among the theclines, show clear evidence for near-even multiples of a chromosome number, either up or down or both within a species or within a genus. So, in addition to the examples mentioned in the results section, *Melanis smithiae* has n = 16 and n = 31, *Emesis mandana* n = 11, 21 and 27. This implies that once one chromosome is either divided into two or fuses with another to form a larger chromosome, then the other follow suit until again a set of chromosomes of about equal size is again attained. Such a concerted evolution of lepidopteran chromosomes was described by Beliajeff (1930) and discussed e.g. by Lorković(1990).

Our riodinid sample sizes are limited, so that it is difficult to say much about the nature of the variation within a species. Several samples from a locality or samples from several localities may have either the same or quite different chromosome numbers.

Table 3. *Haploid chromosome numbers for 17 neotropical lycaenids (Theclinae and Polyommatinae). The genus and species names used in earlier publications are in parentheses.*

Voucher code	Genus	Species	n =	No studied pop./ind.	Locality
Subfamily THECLINAE					
Tribe Eumaeini					
***Eumaeus* section**					
Maeki and Remington 1960, Lyc 11	*Eumaeus*	*childrenae (debora)*	24	2/2	Mexico, OX
de Lesse 1970, M 7		*minyas*	24, ca 45	1/1, 1/1	Mexico, AM
Lyc 1		*minyas* ssp.	23	1/1	DA
***Brangas* section**					
de Lesse 1967	*Evenus (Thecla)*	*coronata*	24	1/2	Ecuador
1367		*regalis*	24	1/1	ES
646		*satyroides*	24	1/1	BA
***Atlides* section**					
Lyc 10		*mavors*	24	1/1	TV
297		*triquetra*	24	1/3	ES
***Thereus* section**					
Wesley and Emmel 1975 (TR-202)	*Arawacus*	*aetolus (linus)*	22–24 (22,23,24)	1/1	Trinidad
de Lesse 1967	*Contrafacia (Thecla)*	*francis*	24	1/1	Argentina
***Strymon* section**					
de Lesse 1967	*Strymon (Thecla)*	*Astiocha (faunalia)*	24	1/1	Argentina
de Lesse 1967	*Strymon (Thecla)*	*eurytulus*	24	1/3	Argentina
Subfamily POLYOMMATINAE					
de Lesse 1967	*Hemiargus*	*hanno*	14	1/1	Argentina
de Lesse 1967	*Itylos*	sp. probably *titicaca* (?)	ca 23–24	1/1	Argentina
de Lesse 1967	*Leptotes*	*andicola*	24, 24–25	1/3	Ecuador
de Lesse 1967		*callanga*	24	1/1	Peru
de Lesse 1967		*cassius*	18	1/1	Argentina

The authors who write about riodinids (Brown 1993a, 1993b; DeVries 1997) stress that there are undescribed species in each major genus and that the genera are in need of revision. We have included in our lists several undescribed species and feel that much of the variation within what we think is a species at a locality may, in fact, be explained through sibling species, each with a different chromosome number. de Lesse (1967) who had studied the relationship of chromosome change and speciation of lycaenids in detail (White 1978; Wiemers 2003) argued that whenever one sees sympatric populations of what seems to be a single species with different chromosome numbers, one must suspect a pair of sibling species with probably, in part, overlapping distributions. On the other hand, species having the same chromosome number may lack an obstacle for interbreeding. Gompert et al. (2006) and Mavárez et al. (2006) have not only shown that hybrid speciation is not only possible but that it has contributed to lepidopteran speciation. It may be argued to be a factor that has stabilized chromosome numbers in two groups with exceptionally stable chromosome numbers: lycaenids and the genus *Heliconius*. Such phenomena are, however, evidently rare. Maeki and Ae (summarized in Ae 1995) have shown that between species crosses involving papilionids with n = 30 show gross chromosome pairing disturbances at meiosis.

Ecology

A coevolutionary process is thought to accelerate the rate of evolution. The riodinids show several kinds of coevolution. They have a potential arms race with their host plants; the larvae of Eurybiini, Lemoniadina and Nymphidiina associate with ants; the representatives of some 16 genera participate in mimicry rings of heliconians, ithomiines, arctiids, dioptids and other lepidopterans (Brown 1993a). In addition to nectar, adults feed on damp sand and mud ('puddling') and carrion (Hall and Willmott 2000). Most riodinid subfamilies and tribes have diverse host plants representing a wide range of both monocots and dicots; in addition the adults

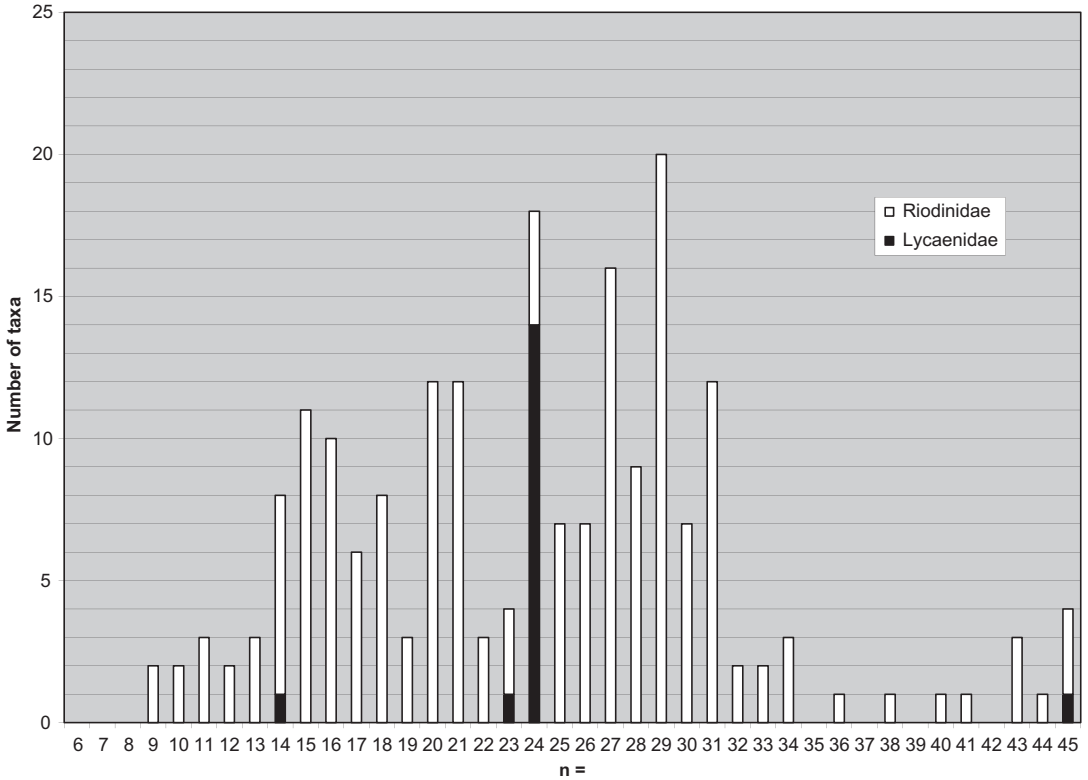

Fig. 1. The distribution of chromosome numbers (the highest numbers are not shown) in Neotropical Riodinids (white columns) and Lycaenids (black columns) shows that Lycaenids have a modal number at n = 24 while Riodinids lack a distinct modal number.

exhibit a variety of food choices and feeding patterns. The extremes are perhaps the Mesosemiini, the known larvae of which all feed on Rubiaceae, and the Riodiniini, that have host plants belonging to at least 15 different families (DeVries 1997). Host plant specialization can certainly drive lepidopteran speciation (Janz et al. 2006).

Population structure

The population structure of lycaenids has received a fair share of attention, since these small butterflies are subject to a major international conservation effort (New 1993). They are easily the most ecologically diversified group of butterflies, ranging from high Arctic to deep tropics. The neotropical blues are widespread and relatively undifferentiated. Many exhibit mass, long-range community dispersal during the dry season like the Coliadinae and Hesperiidae, ranging over the entire region (Robbins and Small 1981; Brown 1993a). In fact, both coliadines and hesperiids have a single distinctive modal number, n = 31 (Robinson 1971), like the lycaenids with n = 24.

In contrast to blues, which are widespread and have reached even the most isolated oceanic islands like Hawaii, the metalmarks are extremely local. They are often geographically well differentiated (Brown 1993a, 1993b), a circumstance that has made them very useful in biogeographic studies (Hall and Harvey 2002b). Riodinid diversity is highest in the Amazon basin, where they make up about 20% of the local butterfly fauna, and decreases rather evenly from there to all directions and up the mountains. According to Hall and Harvey (2002b) the genus *Detritivora* contains a group of species with exceptionally fragmented ranges, so that the *D. (Charis) cleonus* group is made up of nineteen parapatrically distributed species across Amazonia. In addition, the genus *Ithomiola* shows a clear-cut pattern of vertical speciation on mountains, so that there is a series of species with parapatric ranges across an elevational gradient. The younger species are montane and the older ones are found in the lowlands (Hall 2005). Riodinid populations are usually confined to a very narrow microhabitat and the population sizes may fluctuate widely. Some are even nocturnal (Brown 1993a).

Callaghan (1978) seems to be the only author thus far who has studied the population biology of riodinids (*Menandner felsina*). The riodinids exhibit perching behavior as an ingredient of mate choice (Callaghan 1983). Related sympatric species perch at different sites and at different times of the day. This gives rise

to tight niche packing. In addition they use displays and pheromones to discriminate among potential mates. Callaghan (1983) concludes that the set of behaviors and pheromones together have considerable taxonomic value. New (1993, pp. 7–8) points out that perching species tend to have low population densities. Taken together, riodinid populations are small, local and vary in size. These butterflies exhibit behaviour patterns that allow for exact recognition of mates. About 25% of riodinid species have androconial organs, which are highly diverse (Hall and Harvey 2002c). The overall result is a population structure that can be thought to be amenable for chromosome number change, to optimize specific mate recognition and fertilization.

A new chromosome rearrangement resulting in chromosome number change is very unlikely to become fixed in a large, outbreeding population but rather in a small, marginal and isolated one (White 1978, Faria and Navarro 2010). An effective population size that allows for a change is just a few individuals for at least two generations. Under such circumstances the novel karyotype could become fixed in individuals homozygous for it through inbreeding. Alternatively, the situation may persist in the form of a balanced polymorphism. In either case the new karyotype will start to spread. Prezygotic isolation will be built up in addition to the postzygotic one conferred by the novel karyotype, and a new species is born. Chromosomal change need not be the factor that drives speciation (Coghlan et al. 2005). Reproductive isolation can be attained through a variety of mechanisms. Chromosomal change will, if anything, intensify the isolation (Kandul et al. 2007).

Carson (2003) has proposed that mate choice (or non-random mating) will lead to reduced effective population sizes, inbreeding and low gene flow. Riodinids seem to have the potential to exercise mate choice. This, together with their population attributes summarized above, can lead to situations where chromosome changes can be established in populations without necessarily giving rise to hybrids with imbalanced chromosome numbers and concomitant decrease of fitness. We have proposed (Brown et al. 2007a) that chromosome numbers seem to fluctuate in small natural populations of certain scarce or local butterflies.

Now riodinids with their small parapatric ranges and apparently short-lived taxa (but see Hall et al. 2004) represent an ideal example to study the parapatric mode of chromosomal speciation proposed by White (1978). Lukhtanov et al. (2005), Gompert et al. (2006) and Kandul et al. (2007) have shown how the tools of molecular evolution can be tied to study the relationships of chromosome number and speciation in lycaenids. We hope that our study will encourage students of systematics and evolution to take up research on the beautiful and fascinating riodinids of the Neotropics.

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REFERENCES

Ae, S. A. 1995 Chromosome evolution in the Papilionidae. – In: Scriber, J. M., Tsubaki, Y. and Lederhouse, R. C. (eds), Swallowtail butterflies: their ecology and evolutionary biology. Scientific Publishers, pp. 229–239.

Beliajeff, N. K. 1930. Die Chromosomenkomplexe unde ihre Beziehung zur Phylogenie bei den Schmetterlingen. – Z. Abstamm. Vererbungslehre 54: 369–399.

Brown, K. S., Jr. 1993a. Neotropical Lycaenidae: an overview. – In: New, T. R. (ed.), Conservation biology of Lycaenidae (butterflies). Occas. Paper IUCN Species Survival Commission, 8. IUCN, pp. 45–61.

Brown, K. S., Jr. 1993b. Selected Neotropical species. – In: New, T. R. (ed.), Conservation biology of Lycaenidae (Butterflies). Occas. Paper IUCN Species Survival Commission, 8. IUCN, pp. 146–149.

Brown, K. S., Jr., Emmel, T. C., Eliazar, P. J. et al. 1992. Evolutionary patterns in chromosome numbers in neotropical Lepidoptera I. Chromosomes of the Heliconiini (Family Nymphalidae: subfamily Nymphalinae). – Hereditas 117: 109–125.

Brown, K. S., Jr., von Schoultz, B. and Suomalainen, E. 2004. Chromosome evolution in neotropical Danainae and Ithomiinae (Lepidoptera). – Hereditas 141: 216–236.

Brown, K. S., Jr., Freitas, A. V. L., von Schoultz, B. et al. 2007a. Chromosomal evolution of South American frugivorous butterflies in the Satyroid clade (Nymphalidae: Charaxinae, Morphinae and Satyrinae). – Biol. J. Linn. Soc. 92: 467–481.

Brown, K. S., Jr., Freitas, A. V. L., Wahlberg, N. et al. 2007b. Chromosomal evolution in the South American Nymphalidae. – Hereditas 144: 137–148.

Callaghan, C. J. 1978. Studies on restinga butterflies. II. Notes on the population structure of *Menander felsina* (Riodinidae). – J. Lepidopt. Soc. 32: 37–48.

Callaghan, C. J. 1983. A study of isolating mechanisms among Neotropical butterflies of the subfamily Riodininae. – J. Res. Lepidopt. 21: 159–176.

Callaghan, C. J. and Lamas, G. 2004. Riodinidae. – In: Lamas, G. (ed.), Atlas of Neotropical Lepidoptera, Checklist: Part 4A, Hesperioidea and Papilionoidea. Scientific Publishers, pp. 141–170.

Campbell, D. L. and Pierce, E. 2003. Phylogenetic relationships of the Riodinidae: implications for the evolution of ant association. – In: Boggs, C. L., Watt, W. B. and Ehrlich, P. R. (eds), Butterflies/ecology and evolution taking flight. Univ. of Chicago Press, pp. 395–408.

Campbell, D. L., Brower, A. V. Z. and Pierce, E. 2000. Molecular evolution of the *wingless* gene and its implications for the

phylogenetic placement of the butterfly family Riodinidae (Lepidoptera: Papilionoidea). – Mol. Biol. Evol. 17: 684–696.
Carson, H. L. 2003. Mate choice theory and the mode of selection in sexual populations. – Proc. Natl Acad. Sci. USA 100: 6584–6587.
Coghlan, A., Eichler, E. E., Oliver, S. G. et al. 2005. Chromosome evolution in eukaryotes: a multi-kingdom perspective. – Trends Genet. 21: 673–682.
DeVries, P. J. 1997. The butterflies of Costa Rica and their natural history. Vol. II: Riodinidae. – Princeton Univ. Press.
Emmel, T. C., Eliazar, P. J., Brown, K. S. Jr. et al. 1995. Chromosome evolution in the Papilionidae. – In: Scriber, J. M., Tsubaki, Y. and Lederhouse, R. C. (eds), Swallowtail butterflies: their ecology and evolutionart biology. Scientific Publishers, pp. 283–298.
Faria, R. and Navarro, A. 2010. Chromosomal speciation revisited: rearranging theory with pieces of evidence. – Trends Ecol. Evol. 25: 660–669.
Gompert, Z., Fordyce, J. A., Forister, M. L. et al. 2006. Homoploid hybrid speciation in an extreme habitat. – Science 314: 1923–1924.
Hall, J. P. W. 2002. Phylogeny of the riodinid subtribe Theopeina (Lepidoptera: Riodinidae: Nymphidiini). – Syst. Entomol. 27: 139–167.
Hall, J. P. W. 2005. Montane speciation patterns in *Ithomiola* butterflies (Lepidoptera: Riodinidae): are they consistently moving up in the world? – Proc. R. Soc. B 272: 2457–2466.
Hall, J. P. W. and Harvey, J. 2002a. Basal subtribes of the Nymphidiini (Lepidoptera: Riodinidae): phylogeny and myrmecophily. – Cladistics 18: 539–569.
Hall, J. P. W. and Harvey, J. 2002b. The phylogeography of Amazonia revisited: new evidence from riodinid butterflies. – Evolution 56: 1489–1497.
Hall, J. P. W. and Harvey, J. 2002c. A survey of androconial organs in the Riodinidae (Lepidoptera). – Zool. J. Linn. Soc. 36: 171–197.
Hall, J. P. W. and Willmott, R. 2000. Patterns of feeding behaviour on adult male riodinid butterflies and their relationships to morphology and ecology. – Biol. J. Linn. Soc. 69: 1–23.
Hall, J. P. W., Robbins, R. K. and Harvey, J. 2004. Extinction and biogeography in the Caribbean: new evidence from a fossil riodinid butterfly in Dominican amber. – Proc. R. Soc. B 271: 797–801.
Hipp, A. L., Rothrock, P. E., Whitkus, R. et al. 2010. Chromosomes tell half the story: the correlation between karyotype rearrangements and genetic diversity in sedges, a group with holokinetic chromosomes. – Mol. Ecol. 19: 3124–3138.
Janz, N., Nylin, S. and Wahlberg, N. 2006. Diversity begets diversity; host expansions and the diversification of plant-feeding insects. – BMC Evol. Biol. 6:4 doi:10.1186/1471-2148-6-4
Kandul, N. P., Lukhtanov, V. A. and Pierce, N. E. 2007. Karyotypic diversity and speciation in *Agrodiaetus* butterflies. – Evolution 61: 546–558.
de Lesse, H. 1967. Les nombres de chromosomes chez les Lépidoptères Rhopalocères néotropicaux. – Ann. Soc. Entomol. Fr. 3: 67–136.
de Lesse, H. 1970. Les nombres de chromosomes chez les Lépidoptères Rhopalocères en Amérique centrale et Colombie. – Ann. Soc. Entomol. Fr. 6: 347–358.
de Lesse, H. and Brown, K. S. Jr. 1971. Formules chromosomiques de Lépidoptères Rhopalocères du Brésil. – Bull. Soc. Entomol. Fr. 76: 131–137.
Lorković, Z. 1990. The butterfly chromosomes and their application in systematics and phylogeny. – In: Kudrna, O. (ed.), Butterflies of Europe, Vol. 2. Aula Verlag, pp. 332–396.
Lukhtanov, V. A., Kandul, N. P., Plotkin, J. B. et al. 2005. Reinforcement of pre-zygotic isolation and karyotype evolution in *Agrodiaetus* butterflies. – Nature 436: 385–389.
Maeki, K. and Remington, C. L. 1960. Studies of the chromosomes of North American Rhopalocera. 3. Lycaenidae, Danainae, Satyrinae, Morphinae. – J. Lepidopt. Soc. 14: 127–147.
Maeki, K. and Ae, S. A. 1968a. A chromosomal study of seventeen species of butterflies from Hong Kong (Lepidoptera, Rhopalocera). – Kontyû 36: 65–74.
Maeki, K. and Ae, S. A. 1968b. Studies on the chromosomes of Formosan Rhopalocera 2. Pieridae, Lycaenidae and Riodinidae. – Kontyû 36: 124–133.
Mavárez, J., Salazar, C. A., Bermingham, E. et al. 2006. Speciation by hybridization in *Heliconius* butterflies. – Nature 441: 868–871.
New, T. R. 1993. Introduction to the biology and conservation of the Lycaenidae. – In: New, T. R. (ed.), Conservation biology of Lycaenidae (Butterflies). Occas. Paper IUCN Species Survival Commission, 8. IUCN, pp. 1–21.
Pierce, N. E., Brady, M. F. and Heath, A. 2002. The ecology and evolution of ant association in the Lyceanidae (Lepidoptera). – Annu. Rev. Entomol. 47: 733–771.
Robbins, R. K. and Lamas, G. 2004. Lycaenidae. – In: Lamas, G. (ed.), Atlas of Neotropical Lepidoptera, Checklist: Part 4A, Hesperioidea and Papilionoidea. Scientific Publishers, pp. 118–140.
Robbins, R. K. and Small, G. B. Jr. 1981. Wind dispersal of Panamanian hairstreak butterflies (Lepidoptera: Lycaenidae) and its evolutionary significance. – Biotropica 13: 308–315.
Robinson, R. 1971. Lepidoptera genetics. – Pergamon Press.
Stekolnikov, A. A., Ivanov, V. D., Kuznetsov, V. I. et al. 2000. Evolution of the karyotype, wing articulation, male genitalia and the phylogeny of butterflies (Lepidoptera, Hesperioidea, Papilionoidea). – Entomol. Obozr. 79: 123–149, in Russian.
Suomalainen, E. and Brown, K. S. Jr. 1984. Chromosome numbers within *Philaethria* butterflies (Lepidoptera: Nymphalidae, Heliconiini). – Chromosoma 90: 170–176.
Vane-Wright, R. I. 2003. Evidence and identity in butterfly systematics. – In: Boggs, C. L., Watt, W. B. and Ehrlich, P. R. (eds), Butterflies/ecology and evolution taking flight. – Univ. of Chicago Press, pp. 477–523.
Wahlberg, N., Braby, M. F., Brower, A. V. Z. et al. 2005. Synergistic effects of combining morphological and molecular data in resolving the phylogeny of butterflies and skippers. – Proc. R. Soc. B 272: 1577–1586.
Wesley, D. J. and Emmel, T. C. 1975. The chromosomes of neotropical butterflies from Trinidad and Tobago. – Biotropica 7: 24–31.
White, M. J. D. 1978. Modes of speciation. – Freeman.
Wiemers, M. 2003. Chromosome differentiation and the radiation of the butterfly subgenus *Agrodiaetus* (Lepidoptera: Lycaenidae: *Polyommatus*) – a molecular phylogenetic approach. – PhD thesis, Univ. of Bonn.